ETHNOBOTANICAL USES AND STUDIES ON ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ASSOCIATED WITH IMPORTANT TRADITIONAL CROPS OF MANDI DISTRICT

SYNOPSIS

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INTRODUCTION

India holds the second-largest agricultural land in the world. It has 20 agro-climatic regions and 157.35 million hectares of land under cultivation (Directorate of Economics and Statistics, Ministry of Agriculture, India. 2014). Thus, agriculture plays an important role with 58% of rural households depending on it even though India is no longer an agrarian economy. According to Department of Agriculture, Cooperation and Farmers Welfare report estimates that the final estimates for food grain output in 2022-23 stood at a record 329.68 million tonne, up 4% or 14.1 million tons higher than a year ago. However India is self-reliance in food production, its food production between 1947 and 1960 was so miserable that there were risks for the incident of famine.Therefore, the Green Revolution was commenced in the 1960s to increase food production, malnourishment, to feed millions and eliminate poverty in the country. The major crops cultivated in the Green Revolution were rice, sorghum, wheat, maize, barley, and millets (Hall WF, US Department of Agriculture). Agriculture is the main occupation in Himachal Pradesh and is contributing Rs, 1.82 lakh Cores GDP. (Nelson et al. 2019)

Traditional or indigenous crops refer to those that have been historically and culturally most significant to a particular region or society. These can vary widely depending on the location, climate, and culture. But they doesn't give enough yield to feed to the entire population of the nation. These traditional crops of India include several varieties of rice (colored rice, aromatic rice, and medicinal rice varieties), millets, barley, wheat and maize. Millets, cereals, pulses, oil crops is a well-known traditional crops cultivated throughout the world for different medicinal and commercial purpose. Millets, about 6,000 varieties widely grown worldwide for grains and fodder. But the production of millets has reduced and became a fodder crop in just a few decades after the Green Revolution. According to an estimate, there has been a decline of 25.7% in the global area under millets cultivation, whereas a percent increase of 16.26 was noticed in terms of production from 1961 to 2018. Among the continents, the largest reduction in area (90.14%) and production (73.76%) was observed in Europe, followed by Asia (59.79%; 8.77%), whereas the tremendous increase in area and production of millets has been observed in Africa (74.90%; 102.49%). (Sood et.al. 2023).

Millets were the first crop domesticated in Asia and Africa by the local populations, which later on spread across the globe. Among the food crops, millets occupy a relatively lower position in Indian agriculture, though they are really important from the point of food security at the regional and household level. These are a rich source of protein, essential fatty acids,

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dietary fiber, and vitamin B. The health benefits of millet include protecting cardiovascular health, preventing the onset of diabetes, helping people achieve and maintain a healthy weight, and managing inflammation in the gut. It also contains appreciable amounts of dietary fiber and vitamins such as folic acid, vitamin B6, β- Carotene, and niacin. (Nelson et al. 2019). The availability of high amounts of lecithin is useful for strengthening the nervous system. Therefore, regular consumption of millets can help to overcome malnutrition. These are rich in phytochemicals like tannins, phytosterols, polyphenols and antioxidants, They do contain some anti-nutritional factors which can be reduced by certain processing treatments. During Covid-19experts recommending millets as a source of healthy food to boost the immunity and fight covid. (Devi et.al.,2022).Cereals are grown in over 73% of the total world harvested area and contribute over 60% of the world food production providing dietary fibre, proteins, energy, minerals, and vitamins required for human health. (Das et al.2012)Cereals are grown in over 73% of the total world harvested area and contribute over 60% of the world food production providing dietary fibre, proteins, energy, minerals, and vitamins required for human health. (Das et al.2012). Small millets belong to the same family (Poaceae) of major cereals [e.g., rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), and sorghum (*Sorghum bicolor*)], these crops are superior to major cereals in terms of agroecological traits, nutritional quality, and the potential to ensure the immediate demands of food security. These crops are grown in arid- and semi-arid regions where poor soil fertility, limited rainfall, and less attack of insect pests prevail. In addition, these crops are nutritionally dense and, in many adverse environmental conditions, have the potential to diversify diets and overcome food and nutritional shortages for marginal communities. (Muthamilarasan and Prasad, 2021)

One third of global fungal diversity exists in India. The total number of fungal genera reported from world & India is 7,270 and 2,080 respectively. The fungi colonize the root surface, enhance absorption of water and nutrients from the soil and acquire their nutrition by absorption from their surrounding. (Frank,1885).Mycorrhiza (from the Greek words: mykes = mushroom or fungus and rhiza = roots) can be defined as a mutualistic association between plant and fungus localized in a root or root-like structures. The term was coined by Albert Bernhard Frank (1885), which literally means "fungus root". In this association energy moves from plant to fungus and inorganic resources move from fungus to plant (Allen, 1991). More than 90% of vascular plants are found to be mycorrhizal.On the basis of anatomy and morphology, following types of mycorrhiza have been recognised: ectomycorrhiza, endomycorrhiza and ectendomycorrhiza (Harley and Smith, 1983).

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In ectomycorrhiza the root is surrounded by well-developed mantle of hyphae from which arises a network of hyphae called Hartig net which penetrates in between the cortical cells. The mantle can vary widely in thickness, colour, and texture depending on the particular plant fungus combination. Ectomycorrhiza are found on woody plants ranging from shurbs to forest trees (Sharma et al., 2014).

Endomycorrhizas are variable and have been further classified as arbuscular, ericoid, arbutoid, monotropoid and orchid mycorrhizas. Arbuscular mycorrhiza or AM (formerly known as Vesicular arbuscular mycorrhizas or VAM) are mycorrhizas whose hyphae enter in to the plant cells, producing structures that are either balloon like (vesicles) or dichotomously branching invaginations (arbuscules). The fungal hyphae invaginate the cell membrane but do not penetrate the protoplast (i.e interior of the cell). (Harley and Smith, 1983).

Ectendomycorrhizae have the features of both ectomycorrhizae and endomycorrhizae. It has limited occurrence. Ectendomycorrhizal formation induces the growth of short roots, similar to the ectomycorrhizal association in apple seedlings (Thakur and Sharma, 2013).

AM fungi are present in most agricultural and natural ecosystems and play an important role in plant growth, health and productivity hence, they are the most intensively studied types of mycorrhizae (Harley and Smith, 1983; Gianinazzi et al., 1990; Manimegalai et al., 2011). The commonly occurring genera of AM fungi are *Glomus, Gigaspora, Scutellospora, Acaulospora* and *Entrophospor*a. AM fungi forms associations with many shrubs & tree species and not restricted to the particular group of plants. Most of the economically important agronomic grain and forage crops as well as the major commercial fruit, nut trees and berries normally form Endomycorrhizae (Kormanic et al., 1977). The plant root association with these fungi play a vital role in supporting plant's health by improving plant nutrition (Jacoby et al., 2017), suppress pathogen outbreaks (Pieterse et al., 2014), nutrient exchange and modulation of abiotic stress tolerance (Cheng et al., 2019; Baum et al., 2015). They mainly facilitate nutrient uptake, mainly phosphorus, nitrogen (Campo et al., 2020) potassium, sulphur, copper, zinc, calcium etc. (Avio et al., 2006; Fanaei et al., 2015; Prasad et al., 2017; Wang et al., 2018; Liu et al., 2018) and enhance the availability of nutrients as well as their translocation (Rouphael et al., 2015).

The agriculture community is facing a great challenge to enhance the production of traditional crops. A major obstacle is that people are not valuing crops and the potential benefit that can be derived from using them is thus neglected. Furthermore, knowledge is being lost from one generation to the next, with potentially dire implications for long-term sustainable food

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security. It underpins today's production and provides the raw material needed for ensuring continuing supplies tomorrow in the face of rapidly changing world. To meet the challenge, the focus is on to crop production and understand the complexities and interactions of soil biological system and agro-ecosystem as a whole.(Akinola, 2021). There is a great increase in yield per unit land area over past 60 years as a result of increase in energy inputs in terms of mechanization, irrigation, fertilizer and pesticide use. However, these current conventional agricultural practices can not sustain a healthy plant-soil system. Arbuscular mycorrhizal fungi play very important role in soil fertility and plant health. AM fungi are essential for better growth and production of traditional crops successfully. Hence, there is a need to study and document the traditional crops knowledge that has developed and evolved over the centuries suitable to local cultural and socio-ecological systems before it is eliminated completely with the race towards modernisation. The values of traditional food crops and traditional food systems are not explored and documented scientifically barring a few (Bisht et al., 2006; Nautiyal and Kaechele, 2007; Nautiyal et al., 2008). In this context, the present study aims to examine and document the traditional crops and its mycorrhizal association. There is an urgent need to explore, isolate and identify the mycorrhizal association in different traditional crops. Therefore, Researchers are focused on to increased production of traditional crop plants with the help of useful and appropriate soil microbes present in rhizosphere of plants, among them AM fungi are stand out because of their better effects on plant growth.

- Exploration of microbial diversity is primarily important in traditional crops to utilizing these fungi as bio-fertilizer for cultivation of valuable crop plants.
- Considering the important status of crop plants, it is very important to collect, isolate, identify and classify the indigenous AM fungi associated with some traditional crop plants.
- The importance of AMF as bio-fertilizer in the process of production.
- The exploration of predominant AM fungi also helpful for formation of future inoculums as well as its application as fertilizers for production of better seedlings and their survival in adverse conditions.

LITERATURE REVIEW

Traditional or indigenous seeds are those produced, growing or living naturally in a particular country or climate. They are seeds that have been selected and managed by local people in the local growing environment. Traditional crops are grown by local farmers which have their

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own land races of seeds. A variety of crops were grown, including food crops such as wheat, rice, and barley, and non-food cash crops such as cotton, indigo and opium. Millets are not just food, but are an integral part of the culture of thousands of communities all over the country.(Akinola, 2021.Tribal areas in central India and the Himalayas, are the home of millets, where they are the mainstay of agriculture, diet and the cultural systems of the regions. Globally, India is the largest grower of millets, with 26.6% of the world's and 83% of Asia's millet cropping area, followed by Sudan and Nigeria (Meena et al., 2021). The millets commonly grown in India include Jowar (sorghum), Bajra (pearl millet), ragi (finger millet), Jhangora (barnyard millet), Barri (Proso or common millet), Kangni (foxtail/ Italian millet), Kodra (Kodo millet) etc. Millet has wide adaptability to different soil from very poor to very fertile and can tolerate a certain degree of alkalinity. Millets are the ancient crops of the mankind and are important for rainfed agriculture. The best soils are alluvial, loamy and sandy soil with good drainage. Kodo millet can be grown in gravelly and stony soil such as in the hilly region. Proso millet is one of the oldest and is believed to be the first domesticated cereal grain, which was an early introduction to India for extensive cultivation (Sood et.al., 2023).

Mycorrhiza, association of fungus with roots, has been studied throughout the world with reference to their mutualistic symbiosis, patterns of formation, variability in morphological characters and ability of colonizing about 95% of terrestrial plants. Mycorrhizae form mutualistic symbiotic relationships with plant roots of more than 80% of land plants including many important crops and forest tree species (Radhika and Rodrigues, 2010). Arbuscular mycorrhizal fungi (AMF) (Glomeromycota) form an obligate symbiotic relationship with most land plants (Redecker et al., 2013). This symbiotic interaction, consisting of bi-directional transfer of minerals and nutrients between the fungus and plant, is normally mutualistic. AMF are known to enhance the transfer of nutrients of low mobility, especially phosphorus to plants as well as essential mineral nutrients, in exchange for carbon (Smith and Read, 2008). They may also enhance host tolerance to abiotic stresses such as toxic metals (Wang, 2017) and to biotic factors such as plant pathogens (Wang et al., 2020). Functional diversity in AMF can contribute to ecosystem services (Powell and Rillig, 2018). Functional diversity in AMF can contribute to ecosystem. Arbuscular mycorrhizal (AM) fungi have been studied by many researchers due to their agricultural importance to the plants as effective biofertilizers. Though molecular studies revolutionized the traditional taxonomy. still morphological characters are more important for identification. Taxonomy of AM fungi

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is still debatable as research workers have different opinions about their morphology.

International status of the work

Lugo et al., (2003) have studied seasonal variations of colonization, along with its relation to grazing and metabolic host type and fungal spore diversity. Activity of AMF species alone or with various combinations were also studied from the period of establishment of mycorrhiza by Kramer and Wilbur (1949).

Sharif and Moawad (2006) studied the spore density and mycorrhizal colonization in 25 rhizosphere samples of some selected soils and plant roots from fertile and marginal soils of North west Frontier Province of Pakistan. The results showed that VAM spores in soils and root colonization varied in different crops from one site to another under different agroecological conditions. Highest number of mycorrhizal spores was found in potatoes, barley, rice and chickpea in fertile soil and in alfalfa, wheat, barley, oat and grasses in marginal soil.

Song et al., (2015) studied that AMF inoculation does not only promote therapeutic plant growth, but also raises their productivity and amount of chemicals. Therefore, results in an improvement in yield, more especially, of crops, leading to the eradication of hunger cases in various countries.

Kone and Kante (2021) studied the diversity of AMF strains associated to sorghum in Sikasso region soils in Mali. Results revealed 21 AMF morphotypes left between 6 genera (*Glomus, Scutellospora, Gigaspora, Acaulospora, Entrophospora, Sclerocystis*) of three families (*Glomeracea, Gigasporacea, Scutellosporacea*). Four of these morphotypes have been identified.

Germain et al., (2022) reported, 17 genera of AMF belonging to 13 families were identified in all the analyzed corn rhizospheres on the basis of the morphometric characteristics of the spores. However, the family of Glomeraceae represented by the genera *Glomus, Funneliformis, Septoglomus*, and Rhizophagus as well as the families of Acaulosporaceae and Gigasporaceae represented, respectively, by the genres *Acaulospora* and *Gigaspora* are the most abundant.

Fall et al., (2022) studied the morphological diversity of native AMF species associated with the rhizosphere of maize in two locations in eastern Uganda (the Amuria and Serere districts). A total of 19 AMF morphotypes distributed in 7 genera (*Gigaspora, Scutellospora, Glomus,Acaulospora, Archaeospora, Entrophospora*, and *Paraglomus*) are observed. *Glomus* species are abundant in all sites. Maize grown in eastern Uganda is associated with a diversity

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of AMF that could be selected as bio-fertilizers to improve crop production.

Noreen et al.,(2023) studied the AMF diversity in 10 Districts (Charsadda, Mardan, Swabi, Chitral, Dir, Bunner, Haripur, Mansehra, Karak, and Abbottabad) of agroecological importance during Rabi and Kharif seasons of 2019 and 2020 in order to collect different pulse crop plants along with rhizospheric soil. The pulses included French bean (*Phaseolus vulgaris* L.), Mung bean (*Vigna radiata* (L.) R. Wilczek), kidney bean (*Phaseolus vulgaris* L.), peas (*Pisum sativum* L.), soybean (*Glycine max* (L.) Merr.), peanuts (*Arachis hypogaea* L.), and grams (*Cicer arietinum* L.). A total of 21 AMF morphotypes were collected from the rhizosphere of surveyed pulses. On the basis of morphological characterization, they were kept under the following genera, viz., *Glomus, Gigaspora, Sclerocystis, Rhizophagus, Septoglomus, Acaulospora, Diversispora, Claroideoglomus, Archaeospora*, and *Ambispora.*

National status of the work

In India, research on AM fungi is in progress. Agriculture plays a very important in economy mainly in developing countries. It fulfills the requirement of food in entire world. Rural population depends on agriculture, horticulture and control soil erosion and livestock rearing. It generates employment for rural areas (Devi et.al.,2022). India is rich in diversity of fungal species. AM fungi are also reported frequently from various parts of the country. Many new species of AM fungi are reported from India. They are *Sclerocystis sinuosa* (Gerdemann and Bakshi, 1976), *Glomus reticulatum* (Bhattacharjee and Mukerji, 1980). *Gigaspora candida* (Bhattacharjee et al., 1982), *Glomus delhiense* (Mukerji et al., 1983).*Glomus sterilum* (Mehrotra and Baijal. 1992). *Glomus bagyarajii hyderabadensis* (Swarupani et al., 2004).(Mehrotra, 1997). Occurrence of AM fungi in various parts of country was studied by Patil (2005) Pindi et al. (2005), Srinivas et al., (2005).

Daniell et al., (2001) studied the seasonal variation in colonisation which was dominated by *Glomus* species. The decomposition of wheat, oat, barley straw in soil caused a marked increase in the microflora. The oat straw compost yielded significantly more colonies of fungi than either the wheat or barley straw compost. Fungi, mostly *Penicillum* spp., *Mucur* spp. and *Fusarium* spp. were 15 times more abundant in the rhizosphere of wheat roots than rhizosphere of oat, barley or bean root (Tyner, 1948).

VAM fungi are beneficial symbionts for plant growth. They enhance the uptake of nutrients, especially phosphorus (Hayman, 1975), the resistance against pathogens (Davis and Menge, 1981; Graham and Menge, 1982) and increase the absortive surface area of the roots. Inoculation with effective VAM fungal strains alleviate P (phosphorus) deficiency and

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increase plant growth (Carling and Brown, 1982). The *Glomus etunicatum* inoculated maize plants in sandy loam soil, under water stressed conditions, absorbed more phosphorus than non-mycorrhizal plants (Muller and Hofwer, 1991).

VAM fungi colonise most crop and pasture species (Thompson, 1987). For photosynthate, they provide the plant with nutrients, particularly P and Zn (Thomson, 1994). The potential of VAM fungi to enhance plant growth varies greatly. Plants with finely –branched root systems and copious root hairs (e.g. cereals and grasses) generally do not rely heavily on VAM fungi to access and absorb nutrients, while plants with relatively course, poorly branched root systems and few root hairs (e.g. many legumes) may acquire a substantial portion of their nutrient through the actions of VAM fungi (Schweiger et al., 1995).

Goh et al., (1997) in their study found that vesicular arbuscular mycorrhiza contributed to the uptake and translocation of phosphorous (P) and zinc (Zn) to the aboveground plant parts in wheat, especially to the grain and grain yield. VAM play an important role in improving grain quality and quantity in plants by aiding nutrient acquisition in a calcareous soil with low available phosphorous.

Karaki et al., (2004) studied the effect of arbuscular mycorrhizal (AM) fungi inoculation on growth, grain yield and mineral acquisition of two winter wheat (*Triticum aestivum* L.) cultivars grown in the field under well watered and water stressed conditions. Biomass and grain yields were higher in mycorrhizal than nonmycorrhizal plants. The mycorrhizal plants had higher shoot P and Fe concentration than non mycorrhizal plants.

The *Glomus etunicatum* inoculated maize plants in sandy loam soil, under water stressed conditions, absorbed more phosphorus than non-mycorrhizal plants (Muller and Hofwer, 1991). Thakur and Panwar (1995) reported that the arbuscular mycorrhizal fungi inoculation increased the root, shoot and total dry matter production in Mungbean. Sieverding (1991) reported that the cassava, sweet potato, cowpea, soyabean, maize, sorghum, barley, rice, sugarcane, tobacco, cotton, coca, rubber, tea, grasses, and legumes are heavily colonized under the natural conditions. However, wheat, bean, coffee, and tomato are colonized upto a moderate extent by VAM.

Mohammad et al., (1994) studied the response of wheat to VAM fungal inoculate among eroded and non-eroded soil and found the potential role of VAM fungi for improving dryland wheat productivity on eroded soils. 14 AM species were recorded from the agricultural fields of India with only 4-5 species being from the wheat fields (Dalal and Hippalgaonkar, 1995; Singh and Pandya, 1995).

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The importance of the arbuscular mycorrhizal symbiosis contribution to crop productivity depends mainly on the AMF involved (Johnson et al., 1992; Sanders, 2003), on the level of nutrient supply (Liu et al., 2000 a, b), on the conditions of the environment created by cropping practices. Arbuscular mycorrhizal fungi can improve plant growth, especially in dry soil (Auge, 2001). Association of AM fungi with host plant adds in survival strategies as host plant has many advantages as compare to non host plant. AM fungi help host plants by making available nutrients, providing defence against several pathogenic organisms and by influencing the composition of the microflora of the rhizosphere. However, the benefits that AM provides to its host come with a price tag. The plant has to foregoup to 10-20% of its photosynthesis produce to maintain the fungus (Kothmasi et al., 2001).

Carrenho et al., (2002) investigated the influence of peanut (*Arachis hypogea* L.), Sorghum (*Sorghum bicolour* (L.) Moench) and maize (*Zea mays* L.) on the development and diversity of arbuscular mycorrhizal fungi (AMF) from an agrosystem. It was observed that peanut was best plant for promoting the sporulation of AMF, while sorghum favoured the establishment of most AMF species, followed by maize. AMF is a potential natural resource that has an important role in the agricultural system (Finlay 2008) .

Kumar et al. (2003) studied the effect of vesicular arbuscular mycorrhizal fungi inoculation on growth and nutrient uptake of leguminous plants under stress conditions. It was found that the percentage variation in growth rate and nutrient uptake in leguminous plants under drought and different levels of salinity stress conditions were directly proportional to the percentage of mycorrhization.

Most crop plants in temperate agricultural systems build an arbuscular mycorrhiza, therefore, arbuscular mycorrhizal fungi play a key role in nutrition, water relations and pathogens and diseases of crop plants (Jeffries et al., 2003), particularly in organic farming (Gosling et al., 2006).

Sagar and Kaur (2009) isolated 19 species of VAM fungal spores belonging to six genera (*Acaulospora, Endogone, Entrophosphora, Gigaspora, Glomus,* and *Sclerocystis*). Buwalda et al. (2009) studied the importance of mycorrhizal infection for the growth of wheat and barley sown in winter and spring crops. They results that effects of mycorrhizal infection on growth were greater in winter sown than in spring sown wheat.

Bhat et al., (2010) studied the effect of Rhizobium and vesicular arbuscular mycorrhizal fungi on Green gram (*Vigna radiate*) under temperate conditions. There was a significant effect of Rhizobium and VAM on nitrogen levels on nodulation, grain yield, NPK content in grain &

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straw and crude protein content in grain.

Nasrullah et al., (2010) observed the higher AM fungal spore and their root colonization in wheat and maize crops in unfertilized soil. The spores density and infections intensity varies from one site to another under different agro-ecological conditions.

Mader et al., (2011) investigated the effects of the application of a consortium of AMF and plant growth – promoting rhizobacteria (PGPR) on crop yield, grain and soil quality and nutrient uptake of the staple food crop wheat (*Triticum aestivum* L.) in a rotation with either rice (*Oryza sativa* L.) or black gram (*Vigna mungo* L.).

Castillo et al. (2012) studied that in cereals cultivated in Southern Chilean Andosols, arbuscular mycorrhizal fungi play a main role for phosphorous (P) uptake because P acquisition at early stages is crucial for cereals like wheat, barley, and oats.

Ghasemi-Fasaei and Mayel (2012) investigated the influence of arbuscular mycorrhizal inoculation, and P & Zn addition on grain yield and nutrient composition of wheat grown on a calcareous soil. Inoculation with *Glomus mossae* increased both straw and grain yields and also increased P and Zn, Cu and Fe uptakes in wheat tissues.

Singh and Adholeya (2013) studied the diversity and distribution of AM fungi in wheat agro-climatic regions of India. 34 species and 165 isolates of AM fungi were recorded in the 55 study fields scattered in 11 different wheat growing agroclimatic regions of India. They also investigated the role of soil nutrient concentration on the diversity and colonizing ability of VAM fungi.

Naher et al., (2013) studied the symbioses and beneficial effects of arbuscular mycorrhiza fungi (AMF) with tomato (*Solanum lycopersicum*), brinjal (*S.melongena*), potato (*S.tuberosum*), lady's finger (*Abelmoschus esculentus*), cucumber (*Cucumis sativus*), bean (*Phaseolus vulgaris*), pepper (*Capsicum annuum*), wheat (*Triticum aestivum*), aerobic rice (*Oryza sativa*), corn (*Zea mays*), durian (*Durio zibethinus*), rambutan (*Nephelium lappaceum*), pineapple (*Ananas comosus*), citrus (*Citrus sinensis*), banana (*Musa acuminata*), oil palm (*Elaeis guineensis*) and kenaf (*Hibiscus cannabinus*). Among several species of mycorrhiza studied *Glomus mosseae* is well known to colonize several vegetables, fruits, cereals and industrial crop.

Horii and Ishii,(2014) studied the Effect of Arbuscular Mycorrhizal Fungi and Their Partner Bacteria on the Growth of Sesame Plants and the Concentration of Sesame in the Seeds. The results suggest that AMF and their partner bacteria can stimulate the growth and development of sesame plants and increase the content of sesame in the seeds.

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Widiati et al., (2015) revealed his study on Isolation and Identification of Vesicular Arbuscular Mycorrhizae (VAM) in the Rhizosphere of Maize (*Zea mays*) in the Village of Lekopacing, Tanralili District of the Maros Regency. Three mycorrhizal genus were found in the Maize rhizosphere, namely *Glomus, Gigaspora* and *Acaulospora*. More *Glomus* genus was found compares to *Gigaspora* and *Acaulospora*.

Das, (2015) studied the twenty medicinal plants were investigated for Vesicular-arbuscular mycorrhizal (VAM) association in a survey of the Pandam area of Darjeeling Himalaya, West Bengal. Vesicular-arbuscular mycorrhiza was found to be of universal occurrence in almost all plants located at different regions of the globe except a few one under varied eco-habitats. Great variations were found in the VAM infection percentage compared to those at the other sites including seasons also.

Debnath et al., (2016) reveals that ethnobotanical plants are colonized by AM fungi and its association may play an essential role in nutrition of these plants. In this study fifteen ethnobotanical plants were observed for their mycorrhizal structures. All the plants exhibited AM fungal colonization.

Ghosh et.al., (2016) studied the *A. vera* of Aloaceae family growing mainly in hill area of Darjeeling district and plane area of Burdwan district, West Bengal, India. Aloe vera showed 95.5% colonization of VA mycorrhiza. Percentage (%) of vesicle is more than the percentage of arbuscule. Seasonal variation of mycorrhizal colonization of *Aloe vera* has been done. In the season of spring (February-April) the highest colonization percentage had been found. Air-dried rhizospheric soil sample was analyzed for spore population and density. *Aloe vera* is polysporal, different and large number of spores was found and dominant species is *Glomus*.

Kim et al., (2017) said that AMF to improve yield in crops, it is important to note that soil type can affect their association, and therefore, not always that the fungus will promote crop yield. Although AMF colonization is known to benefit plant growth in general, the physicochemical state of the soil may have a direct impact on the plant-fungus symbiotic interaction.

Chanda and Dey,(2018) aimed to estimate and isolate the Arbuscular mycorrhizal (AM) spore population from the rhizospheric soil of some important medicinal plants from the Botanical garden of College campus. All the plants shows very good diversity of rhizospheric soil fungi. Kumar et.al.,(2022) reviwed the total 340 species AMF symbiosis and their potential application in sustainable agriculture.

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Wang, (2018) revealed that AMF are naturally present in several saline soils. The most common species of mycorrhizae observed in extreme saline soils belongs to the genus *Glomus*. *Glomus intraradices*, *G. versiform* and *G. cutunicatum* are the most commonly noticed species of genus *Glomus* in saline environment. The use of arbuscular mycorrhizae in amelioration of salt tolerance has been tested in variety of plants including *Trifolium* spp., *Cucurbita* spp., *Acacia* spp., *Glycine max*, and *Musa* species.

Brar et al., (2019) studied the Effect of different phosphorus level and arbuscular mycorrhizal fungi on yield of green gram (*Vigna radiata*) crop. Fungal populations in the rhizosphere and rhizoplane areas show a qualitative change with age of plants and also with changing environment (Sundar et al., 2011).

Wankhede,(2020) studied the mycorrhizal status and isolation of spores from rhizosheric soil of *Vigna mungo* in Ardhapur region of Nanded District. The % colonization was 70 to 75% in root of *Vigna mungo, Glomus, Aculospora and Gigaspora* spores were identified.

Thoker and Patel,(2020) studied the effect of arbuscular mycorrhizal fungi on growth and Nutrient Status of *Vigna unguiculata* (L.). The results showed that *Glomus hoi* and *Acaulospora kentinensis* significantly increased growth parameters like root and shoot length, total leaf area, fresh and dry weight of roots and shoots in *Vigna unguiculata* L. Black gram (*Vigna mungo* L.) also known as urd bean in India is one of the most important cultivated pulse crops of the '*Vigna*' group and cultivation from ancient times. It is grown in various agro-ecological conditions and cropping systems with diverse agricultural practices, both in rainy (kharif) and post rainy (rabi) seasons. It is grown in summer and winter and accounts for 28 per cent of world grain legume production.

Wendy et al., (2020) revealed 17 AM fungal species recorded from the three ecosystems and belonged to six genera, viz., *Acaulospora* (9), *Rhizoglomus* (1), *Entrophospora* (1), *Claroideoglomus* (2), *Funneliformis* (1) and *Gigaspora* (3). There was dominance of different genera in different ecosystems. The genus *Acaulospora* was abundant in lowlands, genus *Gigaspora* in midlands and the genus *Claroideoglomus* in upland fields.

Mythili et al., (2021) studied the occurrence and distribution of arbuscular mycorrhizal fungi in finger millets growing tracts of Tamil Nadu, India. This study recorded 21 species recorded from the samples were belongs to 11 genera, viz., *Glomus, Claroideoglomus, Paraglomus, Rhizophagus, Funneliformis, Gigaspora, Entrophosphora, Acaulospora, Ambispora, Scutellospora* and *Septoglomus*. The genus*Glomus* was the most dominant genera and *Funneli formis* mosseae was dominant species.

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Chikoti, (2022) Studied the diversity and abundance of AM spores associated with the rhizosphere of *A. odoratissima* affirms that *Glomus mosseae* and *G. aggregatum* were the most dominant, whereas *Scutellospora* spp. were least observed or barely present at two of the experimental sites from where the soil was collected.

Aishwarya et al.,(2022) studied the association of arbuscular mycorrhizal fungi (AMF) with roots and rhizosphere of pea (*Pisum sativum*). A total of 17 AMF fungi belonging to 5 genera 17 species were isolated and identified from the rhizosphere soil. The dominant genus was *Glomus* (6 species), followed by *Acaulospora* (5 species); *Boletus, Gigaspora* (3 species), *Scutellospora* (2 species) and *Sclerocystis* represented by single species. The different microscopic characters like size, colour, details of the wall layers and the nature of their subtending hyphae were also investigated to during this study.

Vishnu et.al., (2023) studied the diversity of VAM fungi in the rhizosphere of 17 medicinal plant species and the parameters, percent root colonization, spore number and diversity of VAM spores associated with the rhizosphere of medicinal plants were calculated. A total of 62 morphotypes belonging to 4 genera, *Glomus, Acaulospora, Sclerocystis* and *Gigaspora* were found along with 2 unidentified genera.

Yadav et.al.,(2023) studied the distribution pattern, diversity, and association of different AMF species with oil-yielding plants in the state of Haryana. The results of the study revealed the percentage of root colonization, sporulation, and diversity of fungal species associated with the selected 30 oil-yielding plants. the presence of several species of different genera of AMF was reported in all the studied oil-yielding plants, that is, 60 AMF belonging to six genera viz. *Acaulospora, Entrophospora, Glomus, Gigaspora, Sclerocystis,* and *Scutellospora* were observed.

RESEARCH GAP IDENTIFICATION

The status of AM association in large number of traditional crops is not yet been studied. There is research paper record available regarding inventory of Meditional plants, some of the millets and pulses. Review of literature shows that no mycorrhizal work has been done intraditional crop plants of District Mandi, and there is no research paper record available regarding inventory of traditional crop plant in mid hill region of Mandi. Results from the current study is important to provide a baseline data about Mycorrhizae and their future applications. Crop diversity is central to food security. It underpins the today's production and provides the raw material needed for ensuring continuing supplies tomorrow in the face of

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rapidly changing world. To fulfill the requirements of ever increasing population, the demands placed on agriculture to supply future food and fiber need have increased. The agriculture community is facing a great challenge to enhance the production. Therefore, to ensure food and nutrition security for our country, it is important to increase the production of these crops and simultaneously revert the control of production, distribution and consumption back to the people. Traditional crops can play a crucial role in sustainable agriculture and make farmers prosperous. So there is need to work more on the following aspects:

- Collection of some traditional crops seed from different area of Mandi district which will include Cereals, Millets crops, Leguminous crops, and Oil seed crop.
- Challenges to production of traditional crops, contribution to nutrition and health, traditional knowledge about the crops.
- Morphological characterization of different traditional crops.
- Assessment of AM fungal spores colonization in different crops.
- Isolation, Identification, and Classification VAM fungal spores from the root adhering soil samples of Major crop plants from field at seedling, Reproductive and maturity stage.

OBJECTIVES

The present work will be carried out with following objectives:

1. To study morphological characters of different Traditional crops and herbarium preperation.
2. To Assess AM fungal spores colonization in different crop plants.
3. To Isolate, Identify, and Classify VAM fungal spores from the root adhering soil samples of traditional crop plants from field at seedling, reproductive and maturity stage.

4. PROPOSED METHODOLOGY

1. Study Area

Mandi is almost at the geographical centre of Himachal, lying along the left bank of the river Beas in the foothills of Shivalik ranges. The district has a total geographical area of 3,950 square kilometers. Topographically, the district can be divided into two main categories, a) Shivalik (Outer Himalaya) region; the Balh valley, Baldwara and Joginder nagar. b) Mid mountain (Inner Himalaya) region: Areas of Chachiot, Karsog, and and Seraj and part of Drang blocks fall in this region. There are 10 Blocks in Mandi, District (Balh, SunderNagar,

DIVISION

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Chachyot, Seraj, Chauntra, Sadar Mandi, Dharmpur, Karsog, Drang and Gopalpur). (Fig.1.1) Mandi is primarily an agricultural district where more than 80% of the total population is directly or indirectly dependent on agriculture. Wheat, maize and paddy are the major cereal crops. Green peas, tomato, ginger, capsicum, potatos are grown in abundance and thus major commercial crops in the district. But farmers do not grow traditional crops so seed collections will be made in different areas of Mandi, district and field trials will be held at Chail chowk in Rabi and kharif season. For crop production sites will be selected in Chailchowk mid hill region, district Mandi.

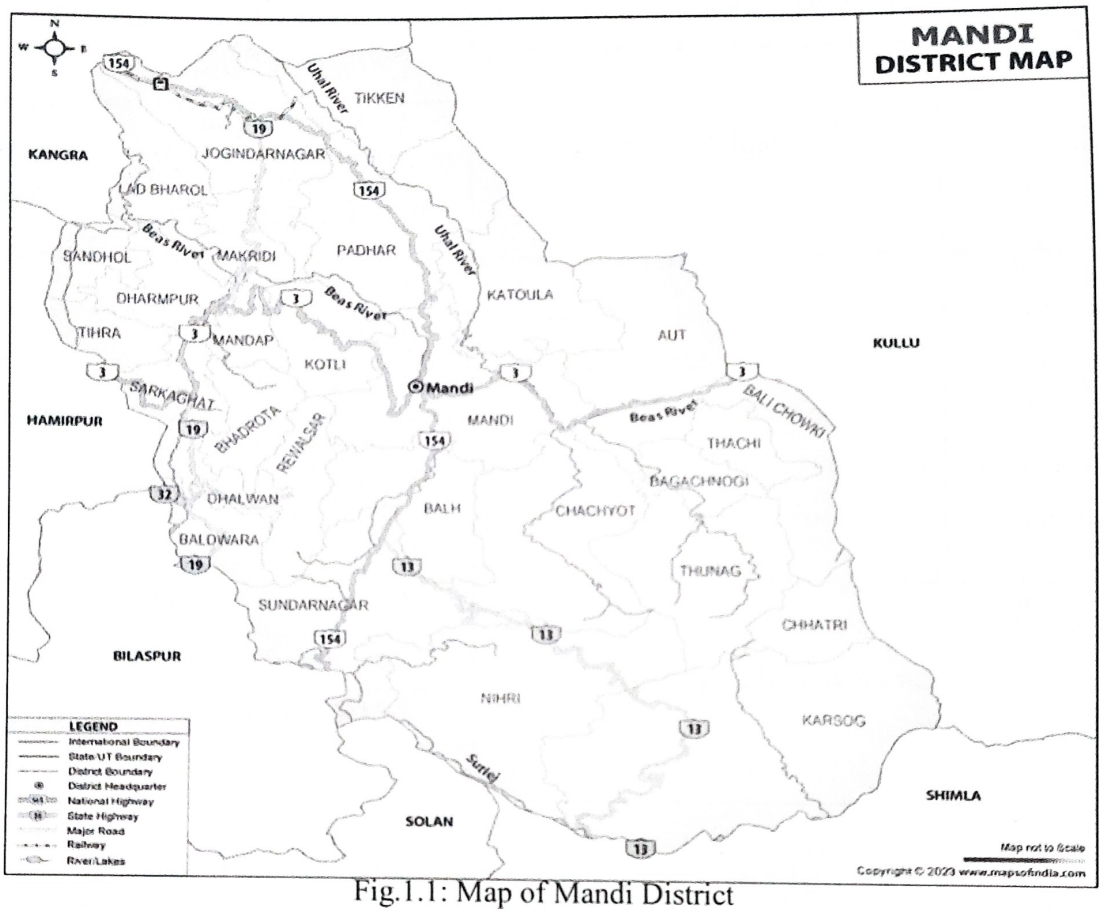

Fig.1.1: Map of Mandi District

2. Materials

For morphological description and mycorrhizal association sample collections will be made during seedling. For morphological description herbarium will be prepared and for mycorrhizal association rhizospheric soils samples of root adhering soil samples and roots of (Cereals and Millets crops, Leguminous crops, and Oil seed crops plant) (Table:1.1) will be

collected from experimental field in Chailchowk mid hill region, district Mandi. The collections will be made during seedling, Reproductive and maturity stage of plant.

Sr. No.	Type of Crop	Name of Crops
1.	Cereal Crops	1. *Zea mays*
		2. *Triticum aestivum*
		3. *Oryza sativa*
		4. *Hordeum vulgare*
		5. *Avena sativa*
2.	Millets crops	1. *Eleusine coracana*
		2. *Setaria italica*
		3. *Echinochloa frumentacea*
		4. *Panicum miliaceum*
3.	Leguminous Crops	1. *Pisum Sativum*
		2. *Vigna mungo*
		3. *Phaseolus vulgaris*
		4. *Cicer arietium*
		5. *Macrotyloma uniflorum*
		6. *lens culinaris*
4.	Oil Seed Crops	1. *Sesamum indicum*
		2. *Brassica compestris*
		3. *Glycine Max*
		4. *Linum usitatissimum*

Table: 1.1 List of Traditional crops found in Mandi, District

3. Methods

3.1 Sampling technique for collecting roots and soil samples from the rhizosphere of Cereals and Millets crops, Leguminous crops, and Oil seed crops plant.

A sterilized trowel (70% alcohol) will be used for scrapping the surface soil. Then the soil will be dig up to a depth of 10-15 cm around the plant. Soil samples will be collected from different depths after removing the top soil i.e 10cm, 15 cm, 20cm below the surface. The samples will be collected and transferred to laboratory in a good quality, air tight and clean

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plastic bags for analysis. These samples will be labelled with paper slip for identifying them. Such samples will be taken from two different sites. Samples will be taken during seedling, Reproductive and maturity stage of plant. The soil will be cleaned, air dried, crushed, homogenized and sieved and stored in air tight bags for further study.

3.2 General laboratory Techniques

Glass wares used

All glass wares used in research work will be of Corning, Borocil, Harco and Tarson.

Cleaning of glass wares

All the glass wares will be washed properly before use under tap water using lab wash. Then these will be soaked in 5% chromic acid for 15-20 min and rinsed properly.

Sterilization

Glass wares will be sterilized in oven at 177 °C for about two hours.

3.3Materials and Tools of Research

Materials used in this study will be soil samples from the rhizosphere of Cereals and Millets crops, Leguminous crops, and Oil seed crops plant, PVLG (polyvinyl alcohol lacto-glycerol), Melzer, sugar, tissue paper, paper labels, clear plastic bags vol. 1 kg. While the tools used will be a hand shovel, analytical scales, scales, a set of soil sieves with the size of 100 µm, 200 µm, 63 µm, stirring rod, scissors, petri dish (diameter of 5 cm, 8 cm, and 10 cm), beaker glass, volumetric flask, centrifuge tubes, spray flasks, object glass, cover slips, micro pipettes, scalpel, spore tweezers, semi-automatic calculation tools, autoclave, a dissecting microscope (Olympus SZ -51, magnification: 0.8-4), compound microscope (Olympus CX31, magnification of 40x - 1000x), Nikon Elipse 80i microscope with camera (magnification 40x-1000x).

4. Physico chemical properties of soil:

For soil analysis, viz., pH, organic carbon, available N, P, K and Bulk density, composite soil samples will be collected from 0-20 cm and 20-40 cm depth from each plot, before transplanting and after harvesting of crops, air dried and sieved to pass a 2mm screen. The pH of soil will be determined using (1:2.5) of soil and water suspension respectively (Jackson, 1973). Organic carbon, available nitrogen, phosphorus and potassium will be determined by using rapid titration method (Walkley & Black, 1934); alkaline potassium permanganate method (Subbiah & Asija,1956); neutral 1N ammonium acetate method (Merwin & Peech,1951), respectively. Whereas, Bulk density (g cm^3) will be estimated by the specific gravity method (Singh,1980).

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5. Preparation of Herbarium

Herbarium will be prepared following the routine practices like collection of the plant samples, pressing and drying followed by poising with 1% of mercuric chloride. After complete drying and posing the plant samples will be mounted on the herbarium sheets (Jain & Rao, 1977). Plant specimens mounted on the herbarium sheets will be identified with the help of herbarium flora of Himachal Pradesh (Chawdhery & Wadhwa, 1984) and herbarium of Career Point University Hamirpur. The collected plants specimens will be deposited to the Herbarium of the Biosciences Department, Career Point University. Botanical name, Local name, Hindi and English name, Family, Taxonomic information, Different uses of the plants will be tabulated.

6. Assessment of VAM infection in roots (Philips and Hayman, 1970)

The root pieces will be thoroughly washed in tap water and boiled at 90o c for 1-2 hours in 10% KOH depending upon the thickness of the roots. The segments after washing in distilled water and will be acidified by immersing in 5N HCL for a few minutes. The root segments will be then stained in 0.05% cotton blue in lactophenol. The segments will be cut into 1cm segments, which will be mounted on slides containing acetic acid: glycerol (1:1 v/v) and the edges will be sealed with DPX mountant. And then roots will be examined under stereomicroscope for mycorrhizal association. (Philips and Hayman, 1970).

7. Assessment of root colonization by Arbuscular mycorrhizal fungi (AMF)

For staining of root to assess root colonization of AMF, a method described by Phillips and Hayman (1970) for roots and modified by Kormanik et al. (1980) will be employed. Freshly collected roots of pea plants will be washed thoroughly with tap water, cut it into 1 cm length and cleared in 10% (w/v) KOH for 1 hour at 90°C, acidified with 1 % HCl and stained with 0.05% trypan blue overnight and then finally de- stained with lactic acid- glycerin (1:1 by volume) at room temperature. Slides will be prepared and observed under a compound microscope for any of structures associated with mycorrhizal fungi viz., hyphae, vesicles or arbuscules. Root colonization will be assessed by using following formula:

$$\% \text{ colonisation} = \frac{Total\ number\ of\ root\ /tissue\ pieces\ examined}{Total\ number\ of\ colonized\ root\ /tissues\ pieces} \times 100$$

8. Methodology for VAM spores isolation and identification

Isolation of VAM spores: There are many methods which can be used for isolating VAM spores from soil. "Wet Sieving and Decanting Technique" (Gerdemann and Nicolson, 1963) will be used in present study. 10g of soil will be taken and mixed in a large beaker until all soil aggregates disperse to leave a uniform suspension. 240µm, 120µm, 100µm, 63µm, 30µm) sieves will be arranged in a descending order. 300um sieve is used for removal of large organic matter. The contents of beaker will be decanted through the sieve. This process will be repeated four and five times. The debris collected on each sieve will be carefully collected in a beaker with the help of level pipe separately for each sieve. The collected debris of each sieve will be filtered separately through Whatman No.-1 filter paper. The filter paper will be observed under the stereo binocular dissecting microscope with the help of microneedles. Each spore will be picked up with the help of a micro needle and will be mounted in Lactophenol to make a semi permanent mount.

9. Diagnostic slide:

The isolated spores will be mounted on glass slide using Polyvinyl Alcohol-Lactic acid Glycerol (PVLG) as mountant. The spores will be then observed for their distinguishing morphological characters such as size, shape, wall characteristics etc., under a compound microscope (100-1000X).

10. Spore Identification:

Spores will be identified according to the manual of identification of AM fungi by Schenck and Perez (1990). The INVAM worksheet will be used for diagnosing the spores. Additional spores not included in the manual will be identified as per the description given in the INVAM website (http://invam.caf.wvu.edu/). The characteristics used in the identification of genus include spore colour, size and shape, spore walls number, colour, thickness and ornamentation, hyphal attachment, shape and type of occlusions, etc.

THESIS OUTLINE:

Chapter1	**Introduction**	This chapter will give the background of the Research topic.

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Chapter2	**Literature Review, Research Gap and Objectives**	In this chapter, the major studies that have been published in research papers and the various methodologies used, will be summarized. Differences will be drawn from these studies and explanations that how research papers contribute to the literature. Research objectives have been decided on the bases of research gap.
Chapter3	**Methodology**	In this chapter, complete details of all the methods, Procedure and techniques will be provided.
Chapter4	**Result and Discussion**	In this chapter, results of the research work will be shown and compared with the previous findings.
Chapter5	**Summary and Conclusion**	In this chapter, the summary of results and future scopes of the further research will be provided.
Chapter6	**References**	References will be provided in Harvard reference System.

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CERTIFICATE
(SYNOPSIS APPROVAL CERTIFICATE)

This is to certify that the Synopsis/Revised Synopsis for Ph.D. Thesis titled, "**_Ethnobotanical uses and studies on Arbuscular Mycorrhizal Fungi (AMF) associated with important Traditional crops of Mandi district ",** submitted by **Ms. Shivani** to the Career Point University, Hamirpur (H.P.), India in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in the Division/Department of **Botany**, School of **Bio Sciences** has been approved by the Research Advisory Committee (RAC) after an Oral Examination of the student on dated 06/01/2024.

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